

Notice of Allowability	Application No.	Applicant(s)
	09/865,291	TSIEN ET AL.
	Examiner	Art Unit
	Rachel K. Hunnicutt	1647

-- **The MAILING DATE of this communication appears on the cover sheet with the correspondence address--**

I claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

- This communication is responsive to the amendment filed November 2, 2004.
- The allowed claim(s) is/are 95,109,115-119,121,125,128,130,131 and 145-162.
- The drawings filed on 24 May 2001 are accepted by the Examiner.
- Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) All b) Some* c) None of the:
 - 1. Certified copies of the priority documents have been received.
 - 2. Certified copies of the priority documents have been received in Application No. _____.
 - 3. Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

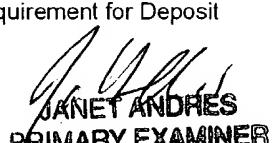
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

- A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
- CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) hereto or 2) to Paper No./Mail Date _____.
 - (b) including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
- DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- Notice of References Cited (PTO-892)
- Notice of Draftsperson's Patent Drawing Review (PTO-948)
- Information Disclosure Statements (PTO-1449 or PTO/SB/08),
Paper No./Mail Date _____
- Examiner's Comment Regarding Requirement for Deposit
of Biological Material
- Notice of Informal Patent Application (PTO-152)
- Interview Summary (PTO-413),
Paper No./Mail Date 1104
- Examiner's Amendment/Comment
- Examiner's Statement of Reasons for Allowance
- Other _____.


JANET ANDRES
PRIMARY EXAMINER

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Jim Fox on November 9, 2004.

The claims have been amended as follows:

95. An isolated polynucleotide encoding a chimeric phosphorylation indicator [having portions comprising a phosphorylatable domain and a fluorescent molecule, where the chimeric phosphorylation indicator comprises, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule, at least one of said donor molecule and said acceptor molecule comprising a fluorescent molecule that has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent molecule,] comprising in operative linkage:

[wherein said donor molecule comprises ECFP (amino acids 1-227 of SEQ ID NO: 6) or a substantially similar variant thereof having at least 80% amino acid sequence identity with ECFP (amino acids 1-227 of SEQ ID NO: 6),] a donor molecule comprising an amino acid sequence having at least 80% sequence identity to ECFP (amino acids 1-227 of SEQ ID NO: 6);

[said] a phosphorylatable domain [comprises] comprising EEEAEYMNMAPQS (SEQ ID NO: 23);

[said] a phosphoaminoacid binding domain [comprises] comprising a Src homology domain-2; and

[said] an acceptor molecule [comprises] comprising [citrine (YFP; SEQ ID NO: 10 having Q69M) or a substantially similar variant thereof having at least 80% amino acid sequence identity with citrine (YFP; SEQ ID NO: 10 having Q69M)] an amino acid sequence having at

least 80% sequence identity to YFP (SEQ ID NO: 10), wherein the acceptor molecule has the mutation K69M with respect to SEQ ID NO: 10,

and wherein the amino acid following the initiating methionine is assigned the '1 position in the numbering of said donor and acceptor amino acid sequences, and wherein at least one of the donor or acceptor molecules comprises a mutation [~~of an amino acid residue~~] which corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 6 or [SEQ ID NO:] 10, wherein said mutation results in a reduced tendency to oligomerize.

147. An isolated polynucleotide encoding a chimeric phosphorylation indicator [~~having portions comprising~~], said indicator comprising:

a phosphorylatable polypeptide; and

~~a fluorescent protein [which polynucleotide is operatively linked to an expression control sequence, wherein said fluorescent protein has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent protein, and wherein said fluorescent protein comprises a green fluorescent protein (GFP) or a fluorescent protein related to a GFP and wherein said fluorescent protein comprises] comprising~~ a mutation which corresponds to an A206K mutation, an L221K mutation, an F223R mutation or an L221K and F223R mutation of SEQ ID NOS: 6 or 10, wherein the amino acid following the initiating methionine is assigned the '1 position in the numbering of SEQ ID NOS: 6 and 10, or an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 2, wherein said mutation results in a reduced tendency to oligomerize, wherein said polynucleotide is operatively linked to an expression control sequence.

151. The kit of claim 150, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise [different] phosphorylatable domains different from each other.

152. The kit of claim 150, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise [different] donor molecules different from each other or acceptor

molecules different from each other or both donor and acceptor molecules different from each other.

Support for the amendment regarding K69M of claim 95 can be found on p. 4, paragraph [0011], p. 18, paragraphs [0056] and [0057]. Support for the amendment regarding the numbering starting at the '1 position following the initiator methionine can be found in U.S. Patent 6,150,176 which is incorporated by reference on p. 38, paragraph [0099] and in Tsien *et al.*, *Ann. Rev. Biochemistry* 67: 509-544, 1998, which is incorporated by reference on p. 19, paragraph [0058].

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rachel K. Hunnicutt whose telephone number is (571) 272-0886. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brenda Brumback can be reached on (571) 272-0961. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

RKH
11/12/04